

Rhodora

JOURNAL OF

THE NEW ENGLAND BOTANICAL CLUB

Vol. 24.

March, 1922.

No. 279.

NOTES ON NEW ENGLAND ORCHIDS,—II.

THE MYCORRHIZA OF GOODYERA PUBESCENS.

OAKES AMES.

(Plates 135 and 136.)

IN a recent paper, entitled Nonsymbiotic germination of Orchid Seeds, Lewis Knudson¹ has published the results of experiments in which he successfully raised seedlings of *Cattleya* and *Laelia* on media from which fungi had been excluded. He proved conclusively that germination of the orchid seeds selected for investigation is not dependent on the invasion of the embryo by a supposedly symbiotic fungal organism, if assimilable food is available. He concluded that germination is a matter of nutrition. It is not necessarily induced by the activities of a fungus. In summing up the results of his experiments he states that the necessity of a fungus for bringing about germination has not yet been conclusively proved, but he does not touch on conditions that seem to be normal among orchids under natural surroundings; in other words, he does not tell us whether germination is possible under natural conditions if the fungus is excluded, and this, it seems to me, is the focal point of the whole orchid mycorrhiza problem.

Although highly interesting from the point of view of laboratory experiments and the possible economic application of the methods employed, it is worthy of note that Knudson's results do not help us to explain the conditions that prevail in nature; his results simply

¹ Bot. Gaz. lxxiii. (1922) 1.

demonstrate that nonsymbiotic germination is possible if by artificial means seeds are supplied with essential food materials in an available form.

Under natural conditions orchids behave in a manner that seems to indicate that the dependence on symbiosis, of a kind, is prevalent. Even under the artificial conditions, that characterize horticultural enterprises, there are indications that symbiosis is essential for successful germination, unless methods such as Knudson described are adopted.

Many years have passed since my interests were centred in the raising of orchid species and hybrids, but it is clear in my memory that seeds sown at the base of a luxuriant orchid plant on a substratum that had become thoroughly settled, gave better promise of germination than those sown on sterilized media in special pots or baskets that had been skilfully prepared and kept under exceptionally clean conditions. And my memory is clear on still another point, that a neighbor who had enjoyed successful horticulture in greenhouses that would have offended, through their rich growth of thallophytes, the carefully trained gardener of a model establishment, raised orchids from seed with uncanny success and inexplicable regularity. When I first read the classic paper which linked Noël Bernard's name indissolubly with symbiosis in the orchids, I imagined that I had at last found a very plausible explanation of the results which I had observed at home and had studied abroad in the realm of orchids raised from seed.

Under natural conditions I have observed that germinating orchids are normally associated with the characteristic fungi of endotrophic mycorrhiza. In fact, as a result of my observations, I have been led to believe that mycorrhiza are not only influential in germination under natural conditions, but that they may have a great deal to do with the manner in which orchids arrange themselves on the ground or on trees and may account for peculiarities of distribution that are well known to collectors in tropical countries and to those systematists who work among orchids. As my observations stimulated interesting conclusions I decided to seek for evidence that would either sustain or demolish my theories.

The species most satisfactory for prolonged and intensive study proved to be our common Rattlesnake Plantain, *Goodyera pubescens* R. Br. (pl. 135). This species is common in New England, is approachable at all seasons of the year and exhibits phenomena that seem to indicate something peculiar in its relation to environment. I undertook to observe this species through a period of twelve consecutive months, my purpose being to follow it from germination of the seed to its adult manifestations.

Goodyera pubescens may well be described as a gregarious species. When it is found under eminently favorable circumstances it forms thrifty colonies in which the leaves almost conceal the underlying humus. Colonies persist for years, and notwithstanding the creeping habit of the rhizomes, retain their compactness. I studied the colony forming habit very carefully and arrived at the conclusion that it is in part to be accounted for by constant replenishment by seedlings. I found that in luxuriant colonies there was always an abundance of young plants that represented several generations of seedlings, and in a paper, published in the *Orchid Review*², I stated that the age of one of these colonies is not to be estimated by the age of its oldest components and that a long-established colony may simply be an aggregation of comparatively young plants, its capacity to attain great age being a direct result of those phenomena which are associated with endotrophic mycorrhiza.

When the mature capsules dehisce in late summer or autumn many of the seeds fall directly to the ground within the confines of the colony and are soon washed by the rain or lodged by other means among the rhizomes and roots of the parent plants. They lie dormant through the winter, and at the beginning of the next growing season germinate and may be observed in July or August, of the year following dissemination, as tiny protocorms³ hardly a millimeter in length (pl. 136, fig. 1.). At this time the testa of the seed is observable, still adhering to the base of the growing embryo. At first, development is very slow. Protocorms devoid of perceptible chlorophyll, without roots or leaves may be found in the autumn. In this condition they are able to pass through the period of dormancy, and at the beginning of the next

² *Orch. Rev.* xxix. (1921) 106.

³ This term was used by Treub to designate a young state in *Lycopodium*. Bernard, *Ann. Sci. Nat.* ser 9, ix. (1909) 9, adopted it for the conical body from which the cotyledon and roots of seedling orchids emerge.

season of active growth are in much the same condition as that in which they entered on their long winter rest. As active growth begins in the spring of the year, roots and leaves develop, (pl. 136, fig. 2) and the little plants soon become large enough to take their place in the colony and rapidly fill in any gaps that may have been caused by the death of older plants or by the creeping apart of their rhizomes. Up to the present time I have been unsuccessful in my search for protocorms beyond the limits of the colonies, and yet within the colonies they are so numerous that the rhizomes, as they elongate, sometimes become entangled.

From these observations I arrived at the conclusion that only those seeds which fall within a colony enjoy a bright prospect of successful germination. This is true because the mycorrhizal fungi are most likely to be present where mature plants are in abundance. Those seeds which drift away on air currents or are blown abroad by the wind and fall where there is no nidus of the necessary fungus, fail to germinate. Otherwise, how account for the colony forming tendency of the species and the peculiarities of distribution?

The protocorms adhere to the roots of mature plants or nestle close to the rhizomes beneath the rosettes of leaves, and may be aptly compared in their appearance to small white spiders at the centre of miniature webs, the webs being the delicate, elongated hair-like structures (rhizoids) which radiate in every direction and serve as passage ways for the fungal hyphae from the interior of the protocorm to the humus (pl. 136, figs. 1 & 3).

My investigations indicate that protocorms are more frequent in close contact with some part of the mature plants than in the open spaces between the rhizomes. That this condition prevails is pretty well shown by the position in which young leafy plants are usually found, that is, in such close proximity to the rhizomes of older plants that they may readily be mistaken for young offshoots. In fact, before I was convinced of the correctness of my conclusions with regard to the nature and origin of young plants that had developed several leaves, I used to suspect that they were really offshoots and that in freeing them for study I had broken them away from the point of origin on mature rhizomes.

It is worthy of note that many protocorms are quite free from direct contact with the surrounding humus. They may lie suspended

by their elongated hairs in the interstices formed by fibrous materials and be perfectly free from particles of organic matter and sufficiently clean to be placed directly without washing, in the killing fluid. In other words, the only means of direct contact with the substratum in such cases is furnished by the hair-like structures. These are rounded at the tip, unicellular, often much longer than the protocorm and produced singly and in tufts from the epidermis.

The hyphae of the fungus penetrate the delicate walls of the hairs (pl. 136, fig. 3) usually at the tip, and enter directly into the humus. In the hairs they may sometimes form structures that suggest fruiting bodies which recall similar structures characteristic of pure cultures of *Rhizoctonia repens* Bernard (pl. 136, fig. 4). Similar structures may also occur within the body of the protocorm, but as I have only observed a single case of this, in a protocorm collected in May 1921, it must be exceedingly rare.

In passing, it may be worth while to suggest that the periodicity of flowering often noted in colonies of *Goodyera pubescens*, and the comparative paucity of flower shoots observed in large colonies of the species, are to be accounted for by the fact that plants of flowering age are few in number, the colonies being made up, for the most part, of young plants which represent several generations.

If we examine the foregoing facts in the light of Knudson's experiments and observations it becomes evident that there are several points which need further elucidation. The questions seem to be: What likelihood is there that the seeds of *Goodyera pubescens* would germinate under natural conditions if mycorrhizal fungi were successfully excluded from the embryos? Is the organic matter, where the plants grow, in a utilizable condition for embryos that are lodged in the humus or suspended above it, or is the embryo incapable of development unless the necessary food substances present in the humus are changed into assimilable organic compounds by the intervention of a symbiotic fungal organism?

I have attempted to show that *Goodyera pubescens* is a gregarious species, that isolated plants are rare, and my observations have indicated that germination of fertile seeds, while a common event within a colony, is perhaps exceedingly uncommon beyond it. Furthermore, I have shown that throughout the early stages of development the protocorms of *G. pubescens* are devoid of chlorophyll and that they

may pass the first growing season and the first winter of dormancy as rootless, leafless whitish bodies. In this condition I have found that they exhibit all the phenomena associated with the presence of endotrophic fungi. It is yet to be proved that without the presence of the fungus they are able to make use of the soluble organic compounds that may be found in the surrounding humus.

The endotrophic fungi of orchid mycorrhiza are made up of closely related species. This is clearly demonstrated by an examination of pure cultures taken from plants of widely separated orchid genera that inhabit different geographical areas. The behavior of the fungus within the orchid and the relation of the orchid to the presence of the fungus is similar in all the species that I have examined. This similarity of behavior and the close structural resemblance that is evident among the species of the fungus, indicates a long association between the fungi and orchids. It is pardonable to suppose that the present day endotrophic fungi of orchids represent very closely what must have been the ancestral form, that is, the form which may have had much to do with the evolution of the orchid family. This assumption is warranted by the fact that despite the vast host of orchid genera and species, there are very few species of endotrophic fungi. It is assumed that the fungus has not changed much since the alliance with the orchids was formed because it has been removed, by its method of life, from those modifying influences that effect an organism that is subjected to the ordeal of competition.

The fungus lives in the protocorm, or in the upper cortical tissues of roots in adult plants, and is capable of luxuriant development. The hyphae do not enter the epidermal cells and are never found in the vascular tract. They may pass through the hair-like organs already described and are therefore able to carry on the functions of the fungus both within and without the body of the protocorm. As the fungus passes from one generation of seedlings to another it is removed from the necessity for reproduction by spores and perhaps, under natural conditions, does not live for any length of time, if at all, as an autonomous organism. When in association with orchids it is supposed to supply food materials in an available form or to render soluble organic compounds assimilable. It surely derives some benefit for its munificence. In *Goodyera pubescens* the fungus lives through-

out the early stages of development in the protocorm. As the protocorm gives rise to the rhizome, and leaves and roots begin to develop, the fungus passes into the cortical cells of the roots. From my observations it does not again invade the tissues of the rhizome. In adult plants invasion of the roots takes place directly from the humus. All the evidence shows that the association is a close one and that both fungus and orchid derive some benefit from it. The capacity of both organisms to thrive, is, it would seem, a sufficient proof that orchid-fungus symbiosis is a well balanced condition. It is true that some of the cells occupied by the fungus frequently exhibit large lobed nuclei, or that a cell may be occupied by two or more nuclei which are formed directly by fragmentation. This may be taken to indicate a pathological condition. Nevertheless, the fungus is digested in the cells farthest removed from the growing point of the protocorm, in tissues that are soon sloughed off as the protocorm elongates (pl. 136, figs. 5 & 6).

Reinheimer⁴ would have us believe that the orchid-fungus association is no longer advantageous, although it is possible that in the past and under special conditions, the fungi have been more useful to the orchids than frequently they are now. If the orchid is capable of nonsymbiotic germination as Knudson and Bernard have demonstrated by laboratory experiments, and the ever present fungus is assumed to be an unnecessary evil in a forced association, sometimes even causing the death of the embryo, how are we to reconcile this assumption with the evidence that may be turned to prove that the orchid family has been eminently successful? The family as at present known is one of the largest phanerogamic groups, numbering some five hundred and fifty genera and fifteen thousand species. In the diversity of floral structure, in the adaptation of the perianth and gynostemium to pollination by insects, and in the extraordinary modification of the vegetative parts to meet the exigencies of terrestrial and epiphytal distribution, the orchids are unsurpassed by any other plant family. Furthermore the Orchidaceae constitute the predominant group as to number of species in some of the richest floral regions of the globe. In Borneo⁵ and the Philippines, for example,

⁴ *Symbiosis, A Socio-physiological Study of Evolution* (1920).

⁵ Merrill in *Journ. Str. Br. Roy As. Soc.* Special No. (1921): *Orchidaceae* 86 genera, 702 species; *Rubiaceae* 64 genera, 333 species; *Euphorbiaceae* 57 genera, 195 species; *Leguminosae* 59 genera, 174 species.

the orchids outnumber in genera and species such successful families as the Rubiaceae, Euphorbiaceae, Leguminosae and Compositae. Is it to be supposed that degeneracy, moribund constitution and maladjustment are just now apparent in the orchid family, or are just beginning to make themselves felt, and that the most highly differentiated concept of the monocotyledons, is on a biological toboggan slide headed for extinction as a penalty for having entered into partnership with a fungus? Surely, if number of genera and species, extraordinary modification of the flower for symbiosis with insects, and wide distribution are to be taken as criteria of biological success, then the orchids have been successful. I am not prepared to admit that orchid-insect symbiosis alone is the fundamental and definitive association that has influenced the evolution of the Orchidaceae and that orchid-fungus symbiosis is simply the result of combat in which orchids have nearly balanced matters and then degenerated.

In the two species of our native orchids of which abundant protocorm material has been available for investigations (I refer to *Goodyera pubescens* and *Spiranthes cernua*), I have sought in vain for protocorms that were free from endotrophic mycorrhiza. Both of these species are prolific in their production of seedlings. In fact I have come to the conclusion that *Spiranthes cernua* is dependent for persistence on numerous progeny. I believe it is a short-lived species and that its replenishment is accomplished by extraordinary fertility and a high degree of success in germination. It is a wide-spread species, adapts itself readily to different conditions of soil and climate, and crosses freely with *Spiranthes gracilis*.

It is assumed that the orchids because of prodigality of seed production are low in organization. Darwin⁶ was puzzled by the vast profusion of seeds produced by the orchids, because, as he put it: "the production of an almost infinite number of seeds or eggs, is undoubtedly a sign of lowness of organization" and, as he went on to say: "that a plant, not being an annual, should escape extinction, chiefly by the production of a vast number of seeds or seedlings, shows a poverty of contrivances or a want of some fitting protection against other dangers." The profusion of orchid seeds has been very well

⁶ The Various Contrivances by which Orchids are Fertilized by Insects 2nd. rev. 277.

indicated by Darwin's observations.⁷ He estimated that a ripe capsule of *Cephalanthera grandiflora* yielded 6,020 seeds, of which very few were bad. In *Orchis maculata* he found about the same number of seeds and estimated that the combined capsules of a single inflorescence would furnish 186,300 seeds, and that, allowing for a certain percentage of infertile seeds, such an inflorescence, if six inches were allotted to each plant produced, had the capacity to populate an acre of land. The great grandchildren of this population, if removed from competition, he found, would be sufficiently numerous to cover, with a uniform green carpet, the entire surface of the earth throughout the globe. The interesting point here, if we accept prodigality of seed yield as a sign of low organization, is not what would be the result if *Orchis maculata* enjoyed one hundred per cent fertility and complete capacity to germinate, but that the species observed by Darwin are admittedly low in the scale of development as we arrange orchids taxonomically, and that they are pretty poor in seed yield when compared with some of the more highly developed species of the *Sarcanthineae*, species which are admittedly the most highly organized orchids from the point of view of botanical classification. In other words it is among the more highly developed orchids, taxonomically speaking, that seed yield reaches truly extraordinary profusion! Fritz Mueller estimated that a single capsule of a *Maxillaria* species yielded 1,756,440 seeds, and yet, *Maxillaria* is admittedly much higher in the evolutionary scale, as botanists estimate development, than either *Orchis* or *Cephalanthera*. But why not admit that though a vast expenditure of energy in the production of seeds was characteristic of the ancestral orchids and indicates that in the dim history of the group a low degree of organization was manifested, the orchids are now a highly specialized concept, the prodigality of seed yield having been perpetuated, and increased in the more highly organized *Sarcanthineae*, as a necessity, because of the peculiarities of the symbiotic relations that sprang into existence when an intrusive fungus became a helpful partner in the economy of the ancestral orchids? In other words, prodigality of seed

⁷ In many species of the *Orchidaceae* the ovules are still rudimentary at the time of pollination; the stimulus exercised by the pollen-tube induces further development. The stimulus is not necessarily dependent on the fertilizing influence of the pollen-tube, as it is brought about when pollen which is without capacity to bring about fertilization is applied to the stigma. This peculiarity has a deep significance in connection with the present discussion.

yield, a million seeds per capsule in some cases, does not indicate a low degree of specialization in a group that depends for successful germination on a symbiotic fungus. For every dozen seeds that fall where endotrophic fungi of the proper type are present, millions must drift to sterile ground and suffer extinction. Consequently in overcoming any economic disadvantages that high yield of seeds implies the orchids through adaptation of seed yield to the exigencies of a symbiotic union have manifested a high degree of success, as success is measured in the human interpretation of organic development. This line of reasoning implies, quite naturally, that orchid-fungus symbiosis is a good thing, that it has been advantageous and admirable. It denies that an association such as orchid-fungus symbiosis is indicative of degeneracy having resulted on the part of the orchid with a consequent moribund constitution. Sparse distribution, notwithstanding an extravagant production of seed, seems to be an indisputable piece of evidence from which to argue that orchids are in a precarious biological situation, but sparse distribution need not be taken as an indication of impending extinction through an unfortunate luxury-symbiosis, if we admit that the orchids are perfectly adapted to sparse distribution, even though incapable of taking possession of the earth.

HARVARD UNIVERSITY, BUSSEY INSTITUTION.

PLATE 135.

GOODYERA PUBESCENS R. BR.

Plant, approximately natural size with flowering and fruiting racemes, detached.

- Fig. 1. Flower much enlarged to show the perianth.
 Fig. 2. Flower sectioned to show relation of the labellum and gynostemium to the ovary, sepals and petals.
 Fig. 3. Gynostemium drawn to show position of stigma and anther.
 Fig. 4. Pollinia.
 Fig. 5. A pollen tetrad.
 Fig. 6. The mature seed

Drawings by Blanche Ames.

PLATE 136.

GOODYERA PUBESCENS R. BR.

- Fig. 1. Protocorm ($\times 25$), showing testa still adherent to the base; spreading hair-like structures through which the hyphae pass to and from the humus, and the growing tip. The darker portion indicates the extent of distribution of the fungus.
 Fig. 2. Four stages in the development of young plants ($\times 2$).
 Fig. 3. The upper portion of a hair-like process through the tip of which two fungal hyphae have passed.
 Fig. 4. Part of a hair-like process in which fruit-like structures have formed.
 Fig. 5. A cell from the lower part of the protocorm showing a large nucleus closely appressed to a mass of digested hyphae.
 Fig. 6. An earlier stage of digestion than that shown in fig. 5. The nucleus and partly digested fungus are surrounded by a skein of hyphae.

Figs. 1-3 from drawings by Blanche Ames. Figs. 4-6 from drawings by the author.